This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 21.

See attached form for additional information. NOV 29 2005 Interagency Report Control

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. **CERTIFICATE NUMBER:** 14-R-0065
CUSTOMER NUMBER: 628

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Tufts University
School Of Veterinary Medicine
200 Westboro Road Bldg. 17
North Grafton, MA 01536

Telephone: (508) -839-7992

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY **(Attach additional sheets if necessarv or use APHIS Form 7023A)**

A. Animals Covered By The Animal Welfare Regulations	**B.** Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	**C.** Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	**D.** Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	**E.** Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reas such drugs were not used must be attached to this report	**F.** TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	0	12	0	12
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	2	0	0	2
7. Hamsters	0	4	5	0	9
8. Rabbits	0	2	10	0	12
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	36	160	56	252
11. Pigs	0	86	45	969	1100
12. Other Farm Animals					
Horse	0	0	24	0	24
Cattle	0	46	14	11	71
13. Other Animals					
Minipigs	0	96	6	0	102
llama	0	0	1	0	1
Mouse-Wild	0	0	0	17	17
Goats	0	0	25	0	25

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and ap Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary inc brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
Executive Officer or Legally Responsible Institutional Official)

(b)(6), (b)(7)c

SIGNATURE (

NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (*Type or Print*)

(b)(6), (b)(7)c

DATE SIGNED 1/23/05

APHIS FORM 7023 (AUG 91) (Replaces VS FORM 18-23 (OCT 88), which is obsolete.)

RaW

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TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University School of Veterinary Medicine
North Grafton, Massachusetts
2005 Annual Report of Research Facility
Registration Number: 14-R-0065

Explanation for Column E:

Calves and piglets were used to produce large numbers of oocysts of *Cyrptosporidium parvum* and other species of *Cryptosporidium* for use in several research programs. Cell culture techniques do not produce significant numbers of organisms necessitating the *in vivo* model for propagation. Four days after oral inoculation, the animal develops diarrhea and begins to shed oocysts. For approximately 10 days during collection of oocysts, the animal may experience discomfort and distress caused by a self-limiting diarrhea. During this time, the animals are closely monitored at least 3 times daily and usually maintain normal appetite, activity levels, and hydration status. Animals may receive fluid therapy to maintain hydration. Animals are euthanized approximately 10 days after the onset of clinical signs as the level of oocyst shedding wanes. Since shedding of large numbers of oocysts is dependent upon the development of diarrhea, antidiarrheal agents and motility modifiers cannot be administered to the animals. Although not expected, animals developing significant signs of distress, including dehydration, depression, or anorexia are euthanized immediately.

Piglets, mice, rats, lab animals, and birds were also used to study various enteric pathogens including microsporidia, cryptosporidia, *Escherichia coli, Clostridium difficile,* and *Candida albicans.* Animals may develop moderate diarrhea after oral inoculation and experience discomfort or distress for the duration of the 3-60 day experiment. Antidiarrheal treatments cannot be administered in order to study the progression of disease but piglets may receive fluid therapy to maintain hydration. Animals are monitored at least 3 times per day and those developing significant signs of distress, including dehydration, depression, or anorexia are euthanized immediately.

Species: Swine Number: 156 Species: Cattle Number: 11

Lamb/kid castration and tail docking is performed by students during the clinical skills course as part of the routine management, husbandry and veterinary practices of the farm. Although it is recognized that lambs/kids probably experience more than minimal or momentary pain and distress, current standard agricultural practices do not utilize anesthetics or analgesics for these procedures. The purpose of this Clinical Skills Course is to train competent veterinarians in the procedures that they will be expected to perform in practice. Since these procedures are expected to be performed without anesthetics and analgesics, exposure to these procedures in veterinary school is critical. All of the current veterinary textbooks and the Sheep Production Handbook produced by the American Sheep Industry Association, Inc. provide instruction on these procedures without anesthesia or analgesia. The "Ag Guide" produced by FASS also allows these procedures to be performed without anesthesia or analgesia prior to 14 days of age.

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In the TCSVM sheep flock and goat heard, these procedures are performed within 7 days of age. These procedures are demonstrated by the instructors and may be voluntarily performed by students. Only one attempt is allowed under the direct supervision of the instructor who intervenes if problems occur. Methods of castration may include elastration, emasculator, and the preferred method, surgical castration. Methods of tail docking may included elastration, emasculator, and hot iron cautery (preferred).
Species: Sheep Number: 56 Species: Goat Number: 0

Piglet castration and tail docking is performed by students during the clinical skills course as part of the routine management, husbandry and veterinary practices of the farm. Although it is recognized that the piglets probably experience more than minimal or momentary pain and distress, current standard agricultural practices do not utilize anesthetics or analgesics for these procedures. The purpose of this Clinical Skills Course is to train competent veterinarians in the procedures that they will be expected to perform in practice. Since these procedures are expected to be performed without anesthetics and analgesics, exposure to these procedures in veterinary school is critical. All of the current veterinary textbooks and the Swine Care Handbook produced by the National Pork Board provide instruction on these procedures without anesthesia or analgesia. The "Ag Guide" produced by FASS also allows these procedures to be performed without anesthesia or analgesia prior to 14 days of age. In the TUSVM Swine Unit, these procedures are performed within 7 days of age. These procedures are demonstrated by the instructors and may be voluntarily performed by students. Only one attempt is allowed under the direct supervision of the instructor who intervenes if problems occur. Methods of castration may include elastration, emasculator, and the preferred method, surgical castration. Methods of tail docking may included elastration, emasculator, and hot iron cautery (preferred).
Species: Swine Number: 813

Wild mice were captured using snap trapping. It is possible that animals may not die immediately when snap trapped, or may be captured by a limb or tail. Accordingly, such an animal may suffer during the 8 overnight hours before traps are checked. Drugs could not be administered during this time because the traps are not attended. Any animal that is not dead when snap traps are checked will be immediately killed by halothane exposure. During the course of the PI's work, he kept a detailed record of the prevalence of such animals, in addition to comparisons of the efficacy of snap traps as opposed to live traps when the two methods are simultaneously used. During the first year, 31 animals were caught by snap trapping and there was no evidence that the animals had not been killed instantly (e.g. trap displaced more than a few inches; animal snapped other than across the spine).
Species: Wild Mice Number: 17

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TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University School of Veterinary Medicine
North Grafton, Massachusetts
2004 Annual Report of Research Facility
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Summary of Exceptions to the regulations and standards:

(b)(6), (b)(7)c

#G663-04 Physiologic Response to Bronchoscopic Lung Volume Reduction Incorporating Fibroblast Growth Factor Type I
#706-05 Definitive Bronchoscopic Lung Volume Reduction (BLVR) Pharmacology/ Toxicology Study
#G717-05 Pneumoreductive Surfactant Repeat Dosing Study
#G718-05 Pneumoreductive Surfactant Dose Distribution Study
#G742-05 Definitive BLVR Pharmacology/ Toxicology Study Using New BLVR Formulation

Exception from *The Guide* housing size recommendations. Sheep recovering from surgical lung volume reduction are housed in the clinical hospital wards to provide the additional post-operative monitoring required. The pens available are slightly smaller than NIH *Guide* recommended size but still allow the full range of normal movement. These sheep may remain in these pens for a period of 2-4 weeks depending on their recovery.

(b)(6), (b)(7)c G631-04 Laboratory Maintenance of the Life Cycles of Ticks and the Pathogens They Transmit

Exceptions from *The Guide* housing size recommendations, cage changing schedules and separation of litters from general cage population. White footed mice are gregarious, particularly during the winter months, and the PI's experience suggest that keeping 4-6 mice (one male and several females) in a shoebox cage or 12-18 mice of mixed sex allows for sufficient breeding with intrinsic regulation of the numbers of offspring

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produced. In this manner, the PI produces only enough for adequate turnover of the colony, but does not experience breeding that needs frequent culling. In addition, the PI finds that cleaning cages less frequently (one cage change every 2-3 weeks) enhances breeding, probably because of pheromone marking of the cage. Finally, although gravid females could be separated, the PI has found that such litters survive less frequently than do those allowed to remain with the other mice.
*No mice were used under this protocol this year.

(b)(6), (b)(7)c #G506-03 Production of Cryptosporidium sp. Oocysts in Calves

Exception from *The Guide* housing size recommendation. Initially calves will be housed in a large pen containing wood shavings. Within 24 hours of arrival at TUSVM, calves will be inoculated orally with Cryptosporidium sp. oocysts. Following inoculation, calves will be monitored daily for fecal shedding of oocysts. Once calves begin shedding oocysts (usually 3-5 days following inoculation), they will be transferred to a free-standing stanchion. These stanchions are 5'x2' with a raised grate flooring to facilitate placement of pans for collection of feces containing the oocysts. Calves typically shed Cyrptosporidium sp. oocyts for up to 2 weeks following inoculation. The adverse effect of the stanchion is that the calf is unable to ambulate freely, but generally, the stanchion is well-tolerated. Non-standard housing (indoors within a free-standing stanchion) is required to facilitate collection of oocytsts present in feces and reducing risk of infection of personnel.

(b)(6), (b)(7)c
#G507-03 Gnotobiotic Piglet Model of E. coli Infection
#G469-02 Immunosuppressed Gnotobiotic Piglet Model of Gastrointestinal *Enterocytozoon bieneusi* Infection
#G432-02 Stx-Specific Human Monoclonal Antibodies for HUS Prophylaxis
#G505-03 Gnotobiotic Piglet Model of Cryptosporidiosis
#G534-03 In Vivo Testing of Anti-Cryptosporidial Compounds
#G485-02 Studies of *Entamoeba histolytica* and *Mycobacterium avium* in Gnotobiotic Piglets
#G685-05 Human Monoclonal Antibody Efficacy Studies in Piglets
#G686-05 Gnotobiotic Piglet Model Microsporidiosis
(b)(6), (b)(7)c
#G559-03 Studies on *Cryptosporidium* Genotypes in Piglet Model of *Cryptosporidiosis*
(b)(6), (b)(7)c
G681-05 Effects of Antibiotics on Stx Production

Exception from *The Guide* housing size recommendations. Piglets are housed for up to 1-8 weeks in gnotobiotic isolators in sectioned pens or individual cages to allow manipulation and monitoring of individual piglets during the study. Piglets are able to move freely and assume normal postures within the limited space. Non-standard housing (in pens/cages within isolators) is required to maintain gnotobiotic status, facilitate collection of oocytsts present in feces, and reduce the risk of infection of personnel.

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(b)(6), (b)(7)c #G668-04 Sustained Coronary Stenosis and Hibernating Myocardium

Lighting and veterinary care. The exemption for the lighting standard and veterinary care for winter woodchucks placed in the hibernaculum will be required. In order to induce the hibernating state, the woodchucks must be in a completely dark, quiet, cool (38-42°F) environment without food during the months of October through February. The majority of animals placed in the hibernaculum will have a telemetry system implanted which will allow us to record ECG, temperature and aortic pressure each day. Hibernation will be confirmed by a recorded heart rate less than 20 beats per minute and a temperature less than 12°C sustained for 2-3 days. Fro the few animals which may not have telemetry system, animals will be considered hibernating if there is no response when blown upon for 2 consecutive visits (2-3 days apart) at which time they will be removed from the experimental studies. No woodchuck will remain in the hibernaculum longer than 40 days. The duration of time spent in the hibernaculum prior to confirmation of hibernation for previous studies has been from 2-5 weeks. The duration to hibernation has significantly decreased following the use of telemetry and improvements in the hibernaculum i.e. quieter cooling unit and low-flow air system.

Additionally, it is also very important for the animals to not feel threatened or a need to defend themselves. Because of this only limited access should be permitted inside the hibernaculum. The animals will be visually examine using a red light and provide fresh water every 2-3 days while they are in the hibernaculum.

In the past, the PI has had good luck with animals entering the hibernating state using these recommended methods. When the woodchucks are in the hibernaculum, they curl up in their hay and are difficult to assess without blowing on them. The hibernating state is very delicate and the woodchucks will not enter hibernation if intruded upon at frequent intervals. The use of large, ceramic water bowls and telelmetry provide both fresh water and objective health status information without unwarranted disturbance which may delay or prevent entrance into the hibernating state and paradoxically increase morbidity, mortality or experimental failure.
*No hibernating woodchucks were used this year.

(b)(6), (b)(7)c
#G540-03 Laboratory Animal Models of *Cryptospooridiosis*
#G680-05 Laboratory Animal Models of *Microsporidiosis*
(b)(5), (b)(7)c
#G689-05 Genetics of *Cryptosporidium parvum*
#G569-03 Propagation of *Cryptosporidium* in Mice
#G748-05 Evaluation of Disinfectants for *Cryptosporidium parvum*

Exception to standard housing. Rodents are housed in collection cages with mesh/wire floors for up to 16 hours/day to allow for the collection of feces.

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(b)(6), (b)(7)c

#G711-05 Nematodes for Immunology Research

Exception to standard housing. Rodents are housed in collection cages with mesh/wire floors for up to 12 hours/day to allow for the collection of feces.

(b)(6), (b)(7)c

G655-04 Polymer Gel to Treat TMJ Pain

The pigs in this study will be exempted from *The Guide* housing recommendation. The pigs will be housed in the Food Animal Ward in the Hospital for Large Animals where the square footage of 4 of the stalls is 22 sq. feet per stall. *The Guide* has minimum guidelines of 24 sq. feet for pigs up to 100kg. The pigs in this study are in the range of 65-75kg.

(b)(6), (b)(7)c

G520-03 Ecology of Tick-Maintained Zoonoses

Exception to standard housing. Animals may be held in live traps for periods of 1-12 hours, depending on time of capture with no source of water.

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Death as an endpoint:

(b)(6), (b)(7)c 590-04 Efficacy Testing of Botulinum Toxin Therapeutics

When systemically absorbed, botulinum toxin is lethal. The purpose of the studies included within this protocol are to evaluate candidate therapeutics for the prevention/treatment of botulism. There are currently no established and recognizable reliable tissue culture systems available for evaluating botulinum toxin activity in vitro. Furthermore, in vivo pre-clinical studies are required prior to human testing of any candidate therapy. Thus, the various murine lethality models are considered to be the "gold standard" for detection of botulinum toxin, evaluation of the effect(s) of botulinum toxin, and evaluation of candidate therapies for the treatment of botulism.

(b)(6), (b)(7)c 541-03 Murine Models of Listeriosis

Mice will be infected with *Listeria monocytogenes* via the intraperitoneal or oral route. These models of murine listeriosis have been described previously by Czuprynski *et al (Infection & Immunity,* 2003, 71:682-689) and Edelson *et al (Journal of Immunology,* 1999, v163:4087-4090). No clinical signs other than being moribound (less likely) or dead (more likely) were described for either of the models described in these articles.

(b)(6), (b)(7)c 542-03 Development of a Conjugate Vaccine for Poultry against *Salmonella enteritidis*

Mice will be infected with Salmonella enteritidis with is a potentially lethal organism for mice.

(b)(6), (b)(7) 665-04 Oral and Intranasal Vaccines against Botulism

When systemically absorbed, botulinum toxin is lethal. The purpose of the studies included within this protocol is to evaluate candidate vaccines for the prevention of botulism. There are currently no established and recognizably reliable tissue culture systems available for evaluation botulinum toxin-activity *in vitro* that correlate with *in vivo* assays. Thus, the murine lethality models are considered to be the "gold standard" for evaluation of the effect(s) of botulinum toxin, and evaluation of candidate vaccines for the prevention of botulism.

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(b)(6), (b)(7)c 729-05 Delineation of Stx and Stx-Hu-MAb Clearance Mechanisms

When systemically absorbed, Stx is lethal to mice. Although none of the studies included within this protocol are intended to induce mortality due to Stx activity, given that the mice will be receiving Stx, there is a possibility that some may die as a result of Stx administration.
11/14/05- Conditionally Approved.

(b)(6), (b)(7)c 689-05 Genetics of *Cryptopsporidium parvum*

Mice will be used for assessing the virulence of recombinant and parent parasite lines and maintenance of parasite lines. One of the measures of parasite virulence is it's ability to kill mice. The purpose of this study is to identify genetic loci affecting or determining the virulence of *C . parvum*. A fundamental premise of this work, is an objective, unbiased method of determining virulence. The ability of *C.* parvum to kill immunosuppressed or deficient mice is, in PI's view, the best measure of virulence. Alternative methods, such as observing symptoms (lethargy, lack of food/water intake, weight loss) are only of use for certain studies, where an approximate, qualitative assessment of virulence is sufficient. Quantitative measures of virulence, as needed in this study, can not be obtained if animals have to be euthanized based on the appearance of certain symptoms. Moving the animals to category C would have a negative impact on the scientific validity of the data obtained from this study Since mice often recover from cryptosporidiosis, even when showing symptoms of severe infection, it is often impossible to predict whether an infection is terminal, or the animals will recover. Currently available in vitro culture systems do not support the sexual phase of the life cycle of C. parvum, or only to a limited extent. In order to study genetic recombination, and the frequency thereof, the parasite has to complete it's life cycle in vivo. In vivo methods do not support, or support only transiently, the production of parasite gametes.

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USDA Registration No. 14-R-0065

Site: 001
Status: Active

Contact Person:

(b)(6), (b)(7)c

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Status: Active

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Site: 001
Status: Active

Contact Person:

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Site: 001
Status: Active

Contact Person:

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Site: 001
Status: Active

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Status: Active

Contact Person:

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Status: Active

Contact Person:

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TUFTS UNIVERSITY

Vice Provost

Physical Address:
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Boston, Massachusetts 02111

November 23, 2005

Dr. Elizabeth Goldentyer
Director, Animal Care Eastern Region
USDA
Eastern Region Office
920 Main Campus Drive, Suite 200
Raleigh, NC 27606-5213

Ref: Customer No. 628
Certificate No. 14-R-0065
Annual Report Submission (APHIS Form7023)

Dear Dr. Goldentyer:

Enclosed please find the above referenced annual report for Tufts University Cummings School of Veterinary Medicine.

Thank you.

Sincerely,

(b)(6), (b)(7)c

Enclosure
C: (b)(6), (b)(7)c

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